

Arizona Iceberg Lettuce Research Council
Research Report
September 1, 2002 – August 31, 2003

Project title: Evaluation and development of biocontrol strategies for lettuce drop in Arizona.

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Cooperating Personnel: Research assistants and technicians from the University of Arizona at Tucson and the Yuma Agricultural Center, Valley Station.

Location of Proposed Research: University of Arizona, Department of Plant Pathology at Tucson, and Yuma Agricultural Center (Valley Station) at Yuma.

Problem and its Significance: Lettuce drop is one of the most common and destructive diseases of lettuce, and occurs in most lettuce-growing regions of the world. In the US, the disease occurs in all major lettuce-producing states including Arizona, California, Florida, and New York. The disease is favored by cool, moist conditions, and in Arizona the incidence of disease is highest during the months of December through early March. At this time, disease incidence may range from a few isolated plants to more than 70% in some fields.

Lettuce drop is caused by two closely related fungi, *Sclerotinia sclerotiorum* and *S. minor*. Both fungi produce hard, durable structures known as sclerotia, which are composed of densely packed, melanized fungal cells. Sclerotia function as survival structures for these fungi, enabling them to persist in soil for extended periods (7-10 years) during unfavorable conditions, and also as disease inoculum in subsequent lettuce crops. Although both fungi cause similar disease symptoms, their ecology is somewhat different. Sclerotia of *S. sclerotiorum* can germinate eruptively, producing infective mycelia, or carpogenically, producing infective ascospores, which can be dispersed by wind throughout the lettuce field and adjacent fields. Sclerotia of *S. minor* only germinate eruptively and disease spread is restricted. Although both fungi are present in lettuce-growing areas of Arizona, *S. sclerotiorum* is the primary species responsible for most cases of lettuce drop in this region.

Present management strategies rely primarily on chemical applications. Currently registered fungicides such as dicloran (Botran), iprodione (Rovral), and vinclozolin (Ronilan) have provided good control of lettuce drop in most situations. For effective fungicide control, the timing of application is critical to provide a chemical barrier between the germinating sclerotia and the developing lettuce plant. However, the efficacy of both iprodione and vinclozolin is significantly reduced after repeated applications. In addition, vinclozolin is scheduled for registration removal. In an effort to reduce losses to lettuce drop, there is a great need for additional disease management strategies. These include the development and evaluation of new fungicides that can be used in rotation with currently registered products. However, with the heightened concern over food and environmental safety, other non-chemical strategies also need to be developed. Biological control offers a promising alternative.

Previous Work: *Sclerotinia* spp. have a very wide host range and cause disease on numerous other agricultural crops. As such, considerable research has been conducted on developing effective biological control strategies for the management of diseases caused by these fungi. Most strategies employ the use of mycoparasitic fungi that specifically attack fungal hyphae or degrade sclerotia. These include *Trichoderma* spp. (5 species), *Coniothyrium minitans*, *Sporidesmium sclerotivorum*, *Gliocladium virens*, *Talaromyces flavus*, *Epicoccum purpurascens*, *Absidia cylindrospora*, *Penicillium citrinum*, and *Pythium oligandrum*, as well as several species or mixture of species of bacteria. Of these, *Trichoderma*, *Coniothyrium*, and *Sporidesmium* have shown the greatest promise.

Trichoderma spp. are perhaps the most widely used mycoparasites and have been employed to control soilborne fungi such as *Phytophthora*, *Rhizoctonia*, *Fusarium*, *Verticillium*, *Botrytis*, and *Sclerotinia* spp. The modes of action of this biocontrol agent include competition with the pathogen for nutrients and space, degradation of pathogen cell walls, suppression of hydrolytic enzymes of the pathogen needed for host penetration, and induction of host defense responses. For control of *Sclerotinia* spp., *Trichoderma* has been successfully used on soybeans, dry beans, chickpeas, green peas, rapeseed, mustard, chicory, basil, sunflower, cauliflower, celery, grape, cucumber, turf grass, and numerous ornamentals. Numerous commercial formulations of *Trichoderma* exist. Some products are composed of a single species or isolate with specific properties, e.g., wide range of target pathogens, unique mode of action, suitability for particular soils, whereas other products are combinations of *Trichoderma* spp. that enhance activity under varying conditions.

Coniothyrium minitans is an effective parasite of sclerotia that are formed by several fungal species such as *Rhizoctonia*, *Botrytis*, and *Sclerotinia*. The primary mode of action of this biocontrol agent is direct colonization of sclerotia resulting in degradation and, in the case of *S. sclerotiorum*, inhibition of apothecia formation and ascospores production. *Coniothyrium* has been used for effective control of sclerotia-forming fungi in onion, bean, pea, rapeseed, carrot, potato, chicory, and caraway.

Field studies were conducted in Arizona in 2001-2002 to evaluate the efficacy of several commercially-available biocontrol agents in reducing the incidence of lettuce drop caused by either *S. sclerotiorum* or *S. minor*. Results from trials using *S. sclerotiorum* as disease inoculum revealed that Contans (*Coniothyrium minitans*) significantly reduced the incidence of lettuce drop by 42.0% compared to controls. In contrast, applications of Rovral significantly reduced the incidence of disease by only 26.0%. Biocontrol products formulated with *Trichoderma* spp., *Sporidesmium sclerotivorum*, or *Bacillus subtilis* (a bacterium) failed to significantly reduce the incidence of disease. In trials using *S. minor* as disease inoculum, Companion (*Bacillus subtilis*) significantly reduced the incidence of disease by 20.4%, compared to controls. Applications of Rovral significantly reduced disease by 30.5% compared to controls, and applications of various *Trichoderma* formulations significantly reduced the incidence of disease by 20.0-5.6%. Applications of *Sporidesmium* had no significant effect on disease incidence. These results demonstrate the successful application of mycoparasitic fungi and/or bacteria for control of lettuce drop caused by either *S. sclerotiorum* or *S. minor*, and suggest that the development of a successful biocontrol program for Arizona lettuce production is attainable.

In these field trials, head weight data was also collected to evaluate the growth enhancing properties reported for several of the commercial biocontrol products. Results from trials using *S. sclerotiorum* as disease inoculum revealed that Contans significantly increased yield (lbs/50 ft of bed) by 73.3% compared to controls. In trials using *S. minor* as disease inoculum, Companion

significantly increased yield by 45.5%, compared to controls. What was particularly interesting was that the use of Rovral resulted in the lowest average head weight compared to all treatments and controls in trials using either *S. sclerotiorum* or *S. minor* as disease inoculum, which had a significant negative impact on yield. This result demonstrates that although Rovral is effective in reducing the incidence of disease, there are potential costs in terms of a reduction in marketable head weight, a consideration which must be taken into account when evaluating the overall benefit of use for any product.

Long-range Objective: The long-range objective of this research is to develop biocontrol strategies for management of lettuce drop in Arizona that may be used independently or in conjunction with standard chemical control strategies. Results obtained in 2001-2002 revealed a significant reduction in disease caused by *S. sclerotiorum* due to the application of one biocontrol product, Contans (*Coniothyrium minitans*), and a significant reduction in disease caused by *S. minor* due to the application of Companion (*Bacillus*) and several *Trichoderma* formulations. If results obtained in 2002-2003 support previous findings, the use of Contans on a commercial basis to control lettuce drop caused by *S. sclerotiorum* could be recommended. In addition, the use of Companion and/or *Trichoderma* products to control lettuce drop caused by *S. minor* could also be recommended.

2002-2003 objectives:

This project is a continuation of AILRC-funded research performed in 2001-2002. Our objective in 2002-2003 is to repeat work performed in 2001-2002 in order to confirm findings from that research.

1. Evaluate the efficacy of commercially available biocontrol products for the control of *Sclerotinia* spp. in Arizona.
2. Evaluate the survival of commercially-available biocontrol agents in Arizona lettuce fields.
3. Evaluate the sensitivity of commercially-available biocontrol agents to standard fungicides used in lettuce production.

Results and Discussion.

Objective 1. Evaluate the efficacy of commercially available biocontrol products for the control of *Sclerotinia* spp. in Arizona.

Trials were conducted at the Yuma Agricultural Center. Experimental design was a randomized complete block design (RCBD) with 4 blocks. Treatments included the biocontrol agents listed in Table 1 in addition to one chemical treatment (Rovral 4F, 1.0 lb a.i./A), one control (no chemical or biocontrol application), and one blank (no chemical or biocontrol application and no disease inoculum). Each treatment plot consisted of 50 ft of bed planted in the lettuce cultivar 'Winterhaven'. Within the trial plot, only every other bed received a biocontrol or chemical applications to fully separate the effect of each treatment.

Two separate sets of trials were conducted with the above treatments: Trials A1 and A2, which utilized *S. sclerotiorum* as disease inoculum, and Trials B1 and B2, which utilized *S. minor* as disease inoculum. Disease inoculum consisted of sclerotia of each fungus produced on sterilized rye grain according to methods described by Matheron and Porchas (1995), and was applied by hand to the surface of each planting bed after lettuce thinning at approximately 4 weeks post-emergence. All treatments were performed in each trial. For each biocontrol treatment, two application schedules were followed. Trials A1 and B1 consisted of a single top-

soil application immediately following planting. Trials A2 and B2 consisted of a top-soil application immediately following planting and a top-soil application immediately following thinning. Product preparation and application rates were as per manufacturer recommendations (Table 1). Furrow irrigation and standard cultural practices for lettuce in Arizona were used for the duration of the trial. At plant maturity, the number of healthy, symptomless lettuce plants per 50' of bed were recorded. In addition, the weight of marketable lettuce heads was recorded to fully assess the total economic benefits from each treatment (disease reduction and growth stimulation).

Results of these studies are presented in Figs. 1-3. In Trial A1 using *S. sclerotiorum* as disease inoculum, a single application of the various biocontrol agents or Rovral did not result in a statistically significant increase in the number of healthy lettuce heads compared to the control (Fig 1). Considering all treatments, Rovral and Contans perform best and equally well. Most of the other biocontrol agents actually resulted in fewer healthy heads than the control. In Trial A2, with two applications of biocontrol agents (and one application of Rovral), Contans performed best, followed by Rovral, and both treatments resulted in a statistically significant increase in the number of healthy heads compared to the control. In Trial A2, most of the other biocontrol agents did not significantly increase the number of healthy heads than the control.

Considerably different results were obtained in Trials B1 and B2 using *S. minor* as disease inoculum. In Trial B1 with a single application of treatment material or in Trial B2 with two applications of treatment material, no treatment resulted in a statistically significant increase in the number of healthy lettuce heads compared to the control, although there was a slight increase for several products (Fig. 1). These results are in contrast with results from 2002 in which several products resulted in a statistically significant increase in the number of healthy heads compared to the control. In 2002, Rovral performed best, followed by Companion, Rootshield, Trichodex, Supresivit, Plantshield, and Soilgard.

What is interesting to note is that in 2002 most products that contain *Trichoderma* or *Bacillus* were moderately effective against *S. minor*, but were ineffective against *S. sclerotiorum*. In 2003, no product, including Rovral, was effective against *S. minor*. In contrast, in both 2002 and 2003, two applications of Contans (*C. minitans*) were moderately effective against *S. sclerotiorum*.

Regarding the effect of treatment on head weight, no significant increases in head weight resulted from the use of any product in a single or double application in Trials A1 and A2 or Trials B1 and B2 (Fig 2). However, in Trials A1 and B1, all products except Rovral resulted in slightly higher head weight compared to the control. In Trials A2 and B2, only Plantshield and Companion (a *Trichoderma* product and a *Bacillus* product, respectively) resulted in slightly higher head weight compared to the control. These results are somewhat contrasting with results from 2002 in which most biocontrol products resulted in higher head weight than the control in four separate trials. Results from 2002 results suggested that there are definite growth enhancing aspects of these products when used in soil infested with *S. minor* (but not *S. sclerotiorum*). Contrasting results obtained from 2003 trials reveal that there may be other undetermined factors (e.g., soil properties, yearly variation in climate, competing soil microflora) that may affect and/or reduce the growth enhancing properties of certain biocontrol agents in specific areas.

Combining data from disease suppression (number of healthy heads or stand/50' of bed) with data from head weight at harvest (lbs/10 healthy lettuce heads), we derived data on yield (lbs of healthy heads/50' of bed). In Trial A1, there were no statistically significant differences in yield among the various treatments and the control, although Contans resulted in the highest

yield (Fig. 3). In Trial A2, with a double application, only Contans resulted in a statistically significant increase in yield compared to the control (Fig. 3). Rovral resulted in the next highest yield followed by Plantshield, but these values were not statistically significant from that of the control.

In Trial B1, Soilgard (a *Trichoderma* product) resulted in a significant increase in yield compared to the control. Supresivit and Contans also resulted in increased yield but the increase was not statistically significant. In Trial B2, no product resulted in statistically significant higher yield compared to the control. However, yield values for three other treatments were higher than that of the control beginning with Contans, and followed by Supresevit and Rovral.

In summary, these trials revealed that Contans was the most effective product for the control of lettuce drop caused by *S. sclerotiorum*. In contrast, no product was particularly effective against *S. minor*, including Rovral.

Objective 2. Evaluate the survival of commercially-available biocontrol products in Arizona lettuce fields.

Soil population densities of each biocontrol agent in fields of Arizona were assessed over the duration of the trials to determine the survival of each agent after application. Soil sampling was conducted prior to planting to assess the natural occurrence of these biocontrol agents. One month after each application, soil samples were again taken to determine how well the biocontrol propagules survived the application process and colonized the plot soil. Soil samples were then taken at harvest to determine the long-term survival of each biocontrol agent in Arizona soil under lettuce cultivation.

To determine soil population densities of *Trichoderma* and *Coniothyrium*, soil dilutions were plated on *Trichoderma* Selective Medium (TSM) and *Coniothyrium* Selective Medium (CSM), respectively, and individual colonies were enumerated. Results for *Trichoderma* enumeration from Trials A and B were very similar and are provided in Fig. 4. *Trichoderma* populations in soil increased dramatically following the applications of Supresevit, particularly with a double application. These results were also obtained in 2002. In addition, *Trichoderma* populations also increased substantially following applications of Plantshield. These results were not noted in 2002, however, a different strain of *Trichoderma* and formulation of Plantshield was provided by the manufacturer for use in 2003. Evidently, this product was superior than the 2002 product in providing viable inoculum that was capable of soil colonization in the Yuma area.

Recovery of *C. minitans* from soil dilutions was not as successful as for *Trichoderma*. Only three soil samples were positive for the presence of *C. minitans* following Contans applications. These samples were from Trial A2 collected on March 6th, and soil populations recovered were 100, 100, and 200 cfu *Coniothyrium*/gram of soil. These values are substantially lower than *Trichoderma* populations recovered which were as high as 13,000 cfu/gram of soil.

Although the use of Supresivit and Plantshield resulted in high colonization of Yuma soil by *Trichoderma*, these products did not perform extraordinary at reducing losses to lettuce drop. These results suggest that the ability to colonize soil to high levels is not a critical factor in assessing the potential of a biocontrol organism at reducing disease. Obviously, some colonization is important, but perhaps only low population levels of highly effective organisms are sufficient for significant disease suppression to be apparent. This fact was evident regarding *Coniothyrium*, which was mostly unrecoverable yet effective in suppressing *S. sclerotiorum*. Several of the manufacturers of the biocontrol products tested in these trials suggested that

sprinkler irrigation following product application may be more effective in promoting colonization of the soil by the biological agent, thereby increasing product efficacy. This irrigation method was evaluated in similar lettuce drop trials conducted in Imperial Valley in 2002-2003 (see discussion below) and will be evaluated in trials conducted in Yuma in 2003-2004.

Objective 3. Evaluate the sensitivity of commercially-available biocontrol agents to standard fungicides used in lettuce production.

To determine if the use of biocontrol agents is compatible with and complementary to standard fungicide applications, the sensitivity of the various commercial biocontrol agents to commonly applied fungicides was assessed in laboratory studies. Evaluations were conducted against Botran, Ronilin, and Rovral in growth inhibition studies. Studies were conducted in Petri dishes containing potato dextrose agar (PDA) amended with the three fungicides at the following rates: 0, 10, 100, and 1000 ppm a.i. Each biocontrol agent was transferred to dishes containing the different fungicides at varying concentrations and incubated for 10 days. Inhibition of fungal growth based upon colony radial diameter was used to determine the relative level of fungicide resistance.

Results of these studies are shown in Fig. 5. Most *Trichoderma* isolates were very tolerant to the three fungicides up to 100 ppm a.i., and most were tolerant at 1000 ppm a.i. The three *Trichoderma* strains used in the formulation of Tusal (a product evaluated in 2003 trials but not 2003 trials) appeared somewhat sensitive to all three fungicides at levels above 1 ppm, which is in contrast to results obtained in 2003. *Coniothyrium minitans* also appeared sensitive to Rovral and Ronilan above 1 ppm, which is also in contrast to 2003 results that showed *Coniothyrium* tolerant to all three fungicides up to 1000 ppm. These conflicting data suggests either resistance to fungicides is a variable character that can be gained or lost during long-term fungal culture or storage in the lab, or the experimental conditions in either 2002 or 2003 were flawed resulting in erroneous results. In both 2002 and 2003 trials, both *S. sclerotiorum* and *S. minor* were quite sensitive to the three fungicides in concentrations over 10 ppm a.i.

These experiments will be conducted again in 2004 in an attempt to resolve the apparent data conflict. The results will be quite important in that they will reveal if chemical and biological control of lettuce drop are compatible disease management strategies and can be used together. In other words, a grower may be able to employ either strategy at the onset of the growing season and switch strategies during the season, if conditions necessitate, without negating the effects of earlier applications. It is known that repeated applications of biocontrol products lead to enhanced performance over time due to the gradual colonization and build-up of soil populations of biocontrol organisms. As such, increased performance by any of the products tested will likely be achieved over several years of repeated usage. If these products are indeed tolerant to fungicides used to control lettuce drop as data generated in 2002 suggests, this would allow growers to begin using these products in advance of an anticipated reduction in fungicide usage and to gradually move into a non-chemical management strategy for lettuce drop while closely monitoring disease management outcomes.

Additional and Future Studies. Additional lettuce drop trials were conducted at the Desert Agricultural Research Center in Holtville, CA in 2002-2003. Most trial parameters including disease inoculum, method of inoculation, treatments, harvest, and disease evaluation were the same as for the Yuma trials. Only two treatments of biocontrol products were evaluated, rather than one and two treatments as were evaluated in Yuma. However, a significant difference in the

Imperial trials compared to the Yuma trials was the method of irrigation, which was applied via overhead sprinklers the entire duration of the Imperial trials.

Results of these trials are provided in Fig. 6. What is immediately evident is that under conditions of sprinkler irrigation, Contans very effectively in controlling lettuce drop caused by *S. sclerotiorum*. In fact, disease suppression was nearly 100%. These results reveal the single most successful control of *S. sclerotiorum* in lettuce reported by any product, chemical or biological, reported in any study to date, and dramatically reveals the potential of *C. minitans* for controlling this destructive disease. Most interesting, there was only slight control of lettuce drop caused by *S. minor*, which is consistent with trials conducted in Yuma in 2002 and 2003. This difference in control efficacy highlights the differences between *S. sclerotiorum* and *S. minor* despite the similarity in fungal biology and the diseases these two fungi cause, and suggests that the eventual development of successful management strategies for these two fungi may in fact involve quite different tactics. What is also important to note is that the efficacy of Rovral against *S. sclerotiorum* and *S. minor* appeared quite similar under the two different irrigation methods, i.e., moderately effective against *S. sclerotiorum* and rather ineffective against *S. minor*.

The different efficacy of Contans exhibited between the Yuma trials using furrow irrigation and the Imperial trials using sprinkler irrigation is most interesting. An additional year of trials is necessary to confirm these findings. These additional trials are planned for 2003-2004 and will involve 1) a repeat of the trials conducted in Imperial using sprinkler irrigation, and 2) a new set of trials conducted in Yuma in which the efficacy of Contans against *S. sclerotiorum* and *S. minor* is compared in trials using either sprinkler irrigation or furrow irrigation. This information will be extremely valuable to growers for projecting product performance under soil and climatic conditions typical to Yuma lettuce production.

Table 1. Application rates for biocontrol products used in lettuce drop trials, Yuma, 2002-2003. Planting date was 10/29/02.

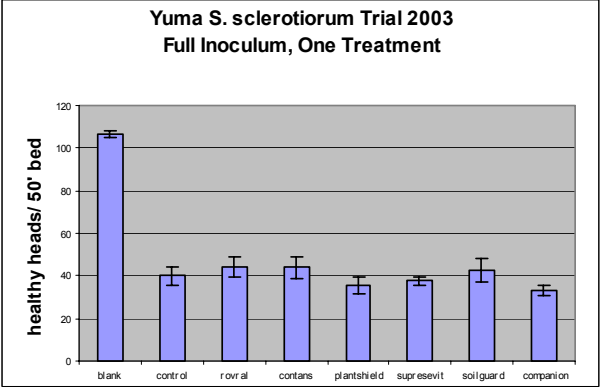
10/29/02	1st treatment application	
	Plantshield	26.25 g/ 0.5 gal/ 100' bed*
	Soilgard	120 g/ 100' bed*
	Supresivit	7.5 g/ 0.5 gal/ 100' bed*
	Trichodex	7.5 g/ 0.5 gal/ 100' bed*
	Contans	3.5 g/ 0.5 gal/ 100' bed*
	Companion	15 ml/ 0.5 gal/ 100' bed

*treatment at 1/2 manufacturer's recommended rate

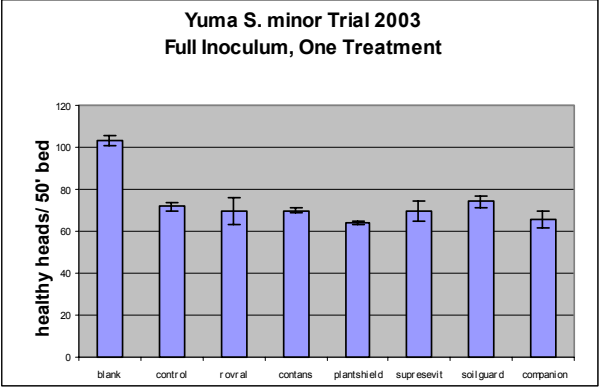
12/18/02	2nd treatment application	
	Plantshield	26.25 g/ 0.5 gal/ 50' bed
	Soilgard	120 g/ 50' bed
	Supresivit	7.5 g/ 0.5 gal/ 50' bed
	Trichodex	7.5 g/ 0.5 gal/ 50' bed
	Contans	3.5 g/ 0.5 gal/ 50' bed
	Companion	7.5 ml/ 0.5 gal/ 50' bed
	Rovral	1 lb a.i./acre

Fig. 1. Disease incidence by trial

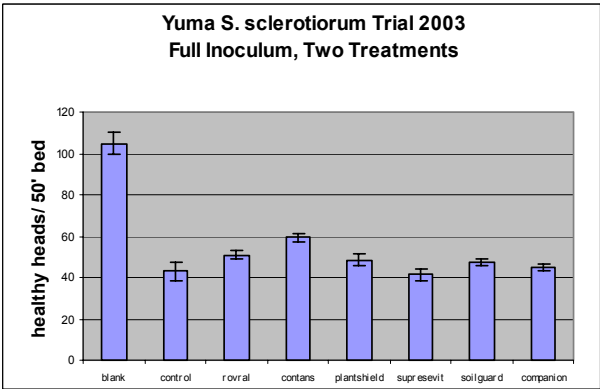
Trial A1



Trial B1



Trial A2



Trial B2

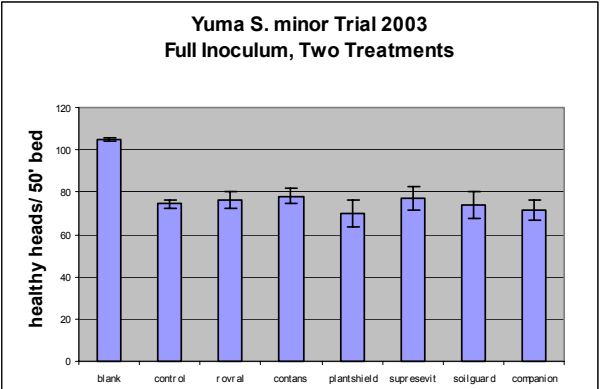
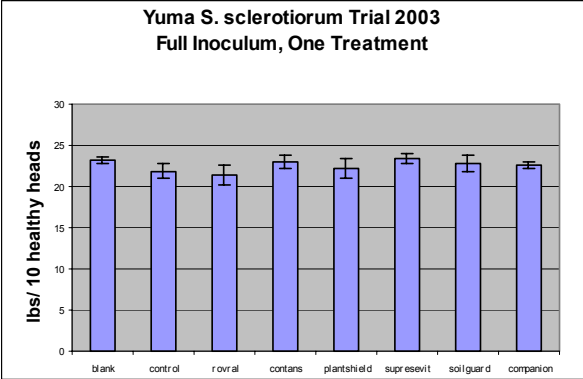
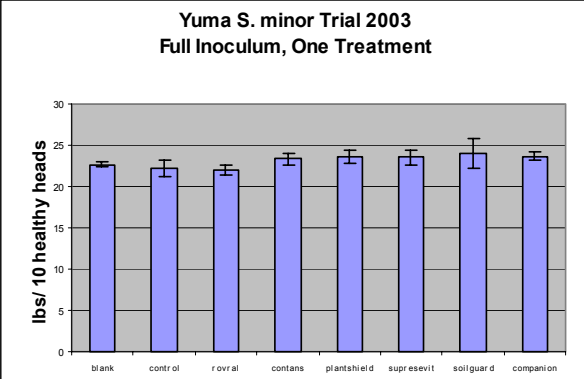


Fig. 2. Head weight by trial

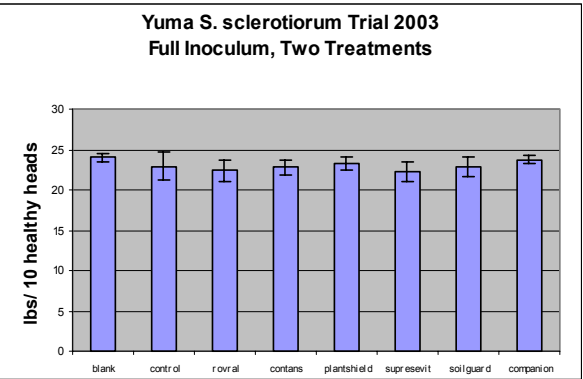
Trial A1



Trial B1



Trial A2



Trial B2

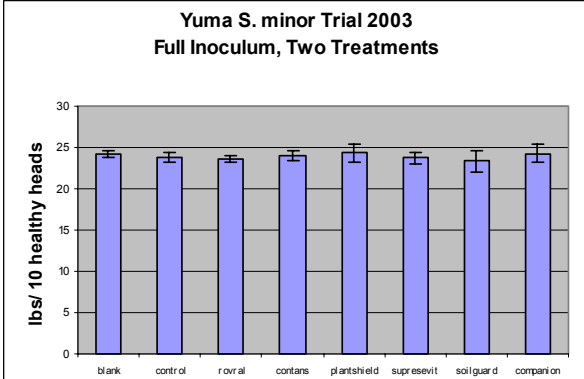
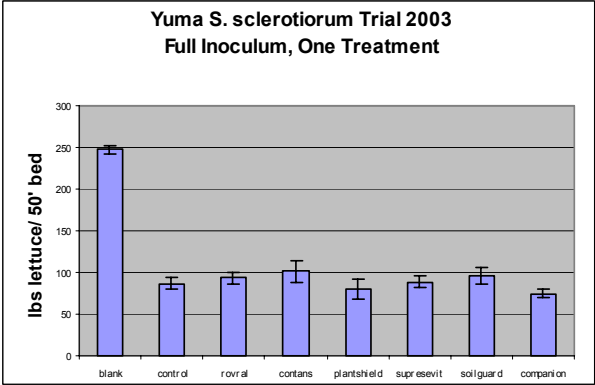
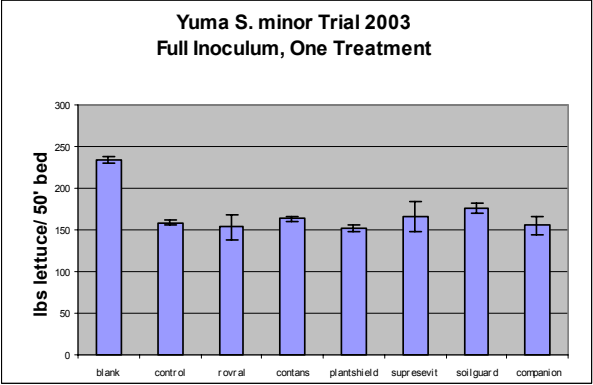


Fig. 3. Yield by trial

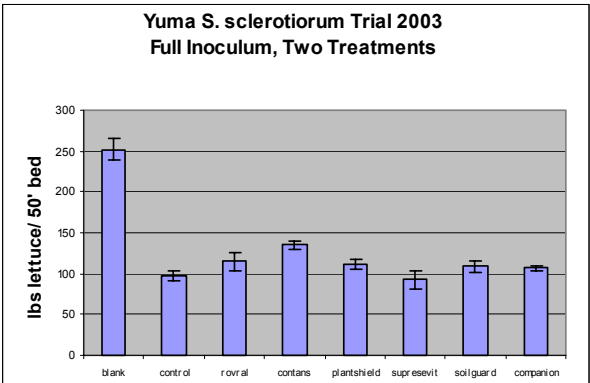
Trial A1



Trial B1



Trial A2



Trial B2

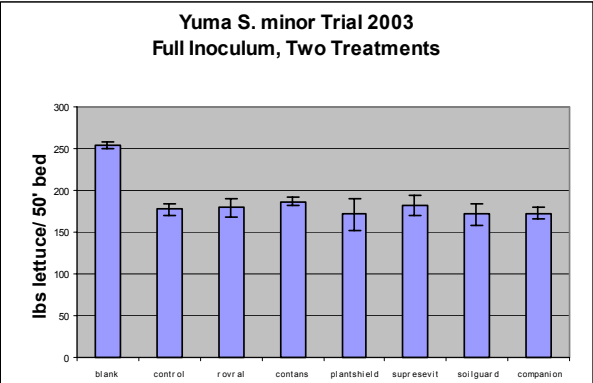
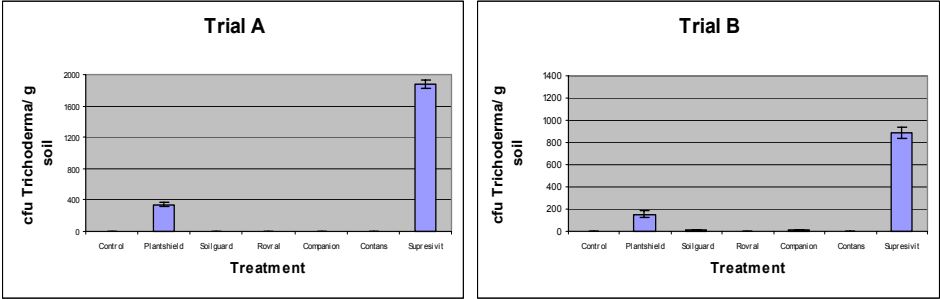
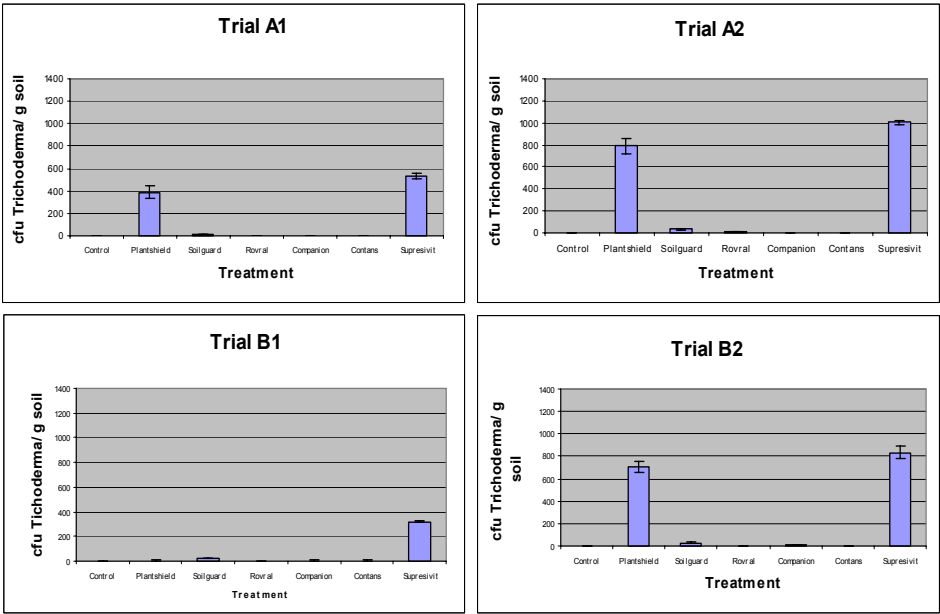


Fig. 3. *Trichoderma* soil populations by trial and by date

Dec 18,



Feb 6,



March 6,

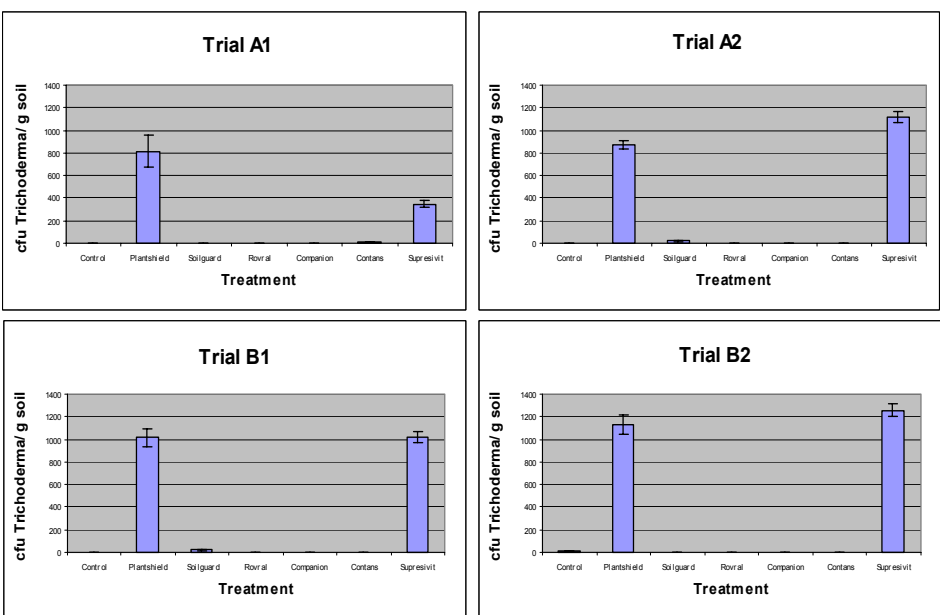


Fig. 5. Tolerance of biocontrol agents and *Sclerotinia* spp. to fungicides.

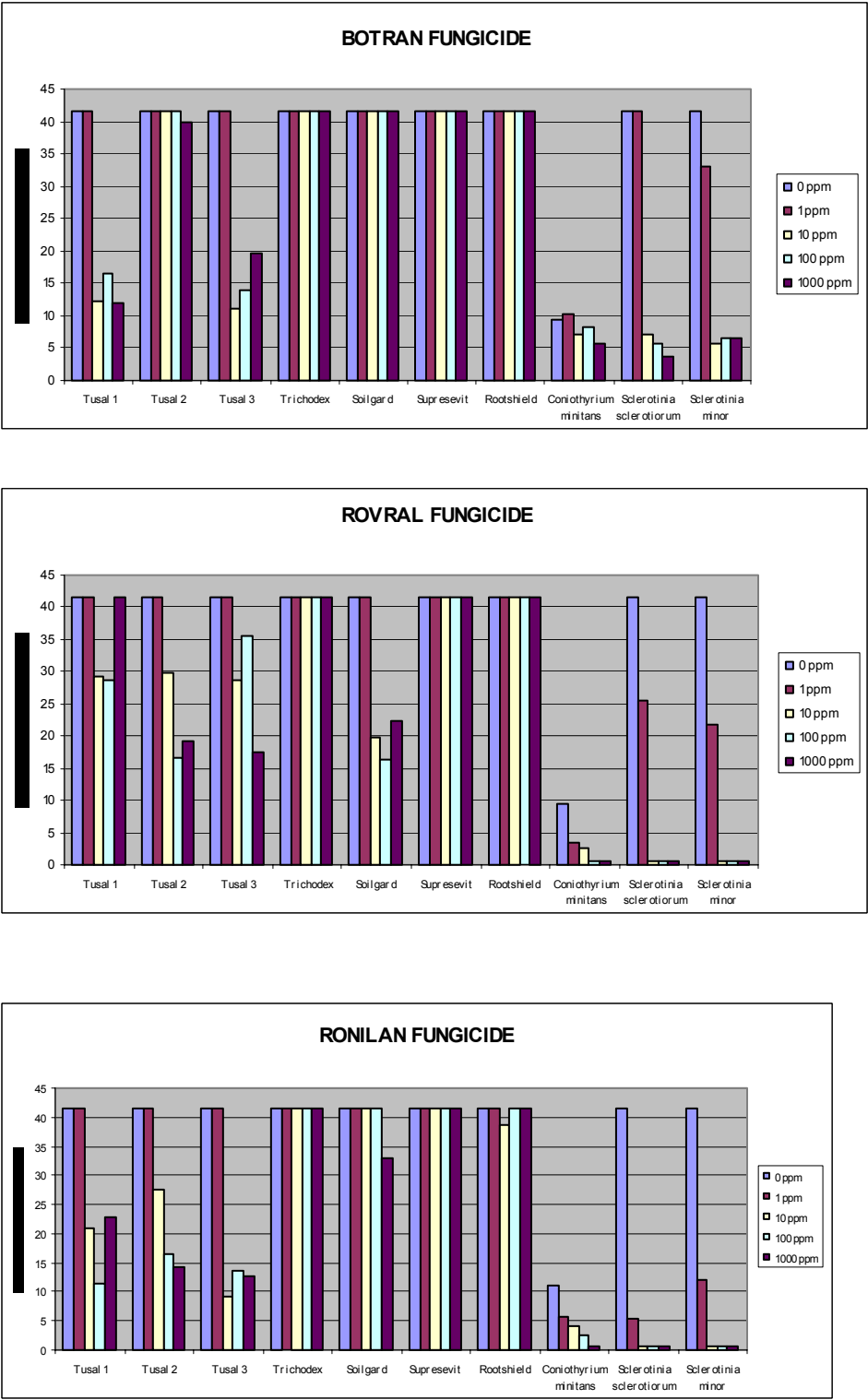


Fig. 6. Disease incidence and yield by trial, Imperial Valley, 2003

